STUDIES ON POST HARVEST MICROBIOLOGY OF VEGETABLES AND FRUITS

DISSERTATION

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CERTIFICATE

This is to certify that the dissertation entitled **STUDIES ON POST HARVEST MICROBIOLOGY OF VEGETABLES AND FRUITS** submittd in partial fulfilment of the requirements for the award of the degree of M.Phil. (Agriculture) in Microbiology of Agriculture Centre, Aligarh Muslim University, Aligarh is a bonafide record of the research work carried out by **Mrs. SHASHI SHARMA** under my supervision and guidance. No part of the dissertation is submitted for any other degree or diploma. The assistance and help rendered during the course of this investigation and sources of literature are duly acknowledged.

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Sharma,

(SHASHI SHARMA)

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INTRODUCTION

Vegetables and fruits are natural sources of proteins, carbohydrates, vitamins and minerals, therefore, occupy an important position in human diets (Work & Carew, 1955). These are highly perishable commodity and liable to damage both in the field and after harvest upto the consumers. In the later, more damage is done due to primitive and defective methods of handling and storage. The damages to fruits during post harvest are no less as compared to pre harvest losses due to diseases (Sudhir Chandra, 1986). Post harvest losses could be due to physical, physiological factors and to pathogenic microorganisms. The physical factor include the mechanical injuries , while physiological factors the response to post harvest environment. The microbiological deterioration and spoilage of fruits is the third category, ofcourse the interactions between the three are more damaging (Sudhir Chandra, 1986).

Pimentel (1983) estimated that 10-21 percent losses occur after harvest, Eckert (1978) enumerated the following consequences of post-harvest deterioration of perishables :

1. Reduced post harvest life of the product due to accelerated ripening or senescence triggered by ethylene released from a few diseased fruits in a package or a storage room.

2. Possible contamination of the edible product with a mycotoxin elaborated by the disease inducing microorganisms, e.g. patulin produced by Penicillium

expansum in diseased apples (Sommer et al. 1974) or furanoterpenoid metabolites in sweet potatoes infected with Ceratocystis fimbriata (Boyd, 1972).

3. Softening of processed fruits by heat tolerant macerating enzymes such as those secreted by Rhizopus stolonifer in incipient infections on peaches.

According to Smith et al. (1964) there are more than 250 known parasitic diseases of fruits that cause decay and blemishes during transit, marketing and storage. In India precise data on losses are not available but it is estimated that the average loss of fruits during post harvest periods is at 20-30% (Mehta et al., 1975a). About 107 million metric tonnes of total food produce are lost annually (FAO estimates) with high percentage in tropical countries. A loss of Rs. 200 crores annually has been estimated (only in respect of fruit) due to post harvest diseases including storage and transit in India.

Ratnam and Nema (1967) while assessing losses from various stores and markets observed considerable losses on apples (12.4-26.7%), banana (15.3-26.7%), citrus (14.05-23.2%), mango (23.3-29.8%), pear (10.32-23.4%), tomato (16.6-31.0%). The losses were more during August (23.05%), September (26.72%). While Thakur and Chenulu (1970) observed a single peak of high intensity of disease during the month i.e. June-July.

According to Mandal and Das gupta (1981) perishable fruits and vegetables suffered an overall loss of greater than 25%. They divided the fruits into 2 categories on the basis of damage.

(a) Worst sufferer (above 10% loss) are mango, pomegranate, apple, pear, mandarian orange, sweet orange, kagzi lime, litchi, grape, pumpkin, bittergourd, brinjal, tomato, chilli, potato, garlic and ginger.

(b) Medium sufferer (5-10%) include papaya, pineapple, banana, guava mango, cucumber, cabbage, cauliflower, radish, carrot.

Mandal and Dasgupta (1981) also reported that most of the pathogens affecting fruits are polyphagous in nature but some are most specific such as Absidia corymbifera on mandarin orange, Botryosphaeria ribis on pear; Penicillium purpurogenum on mango.

Mandal (1981) found that pineapple, mandarin orange, grape, tomato, pointed gourd need immediate transportation as they are more liable to damage. The refrigerated fruits and vegetables also need fast transport as they suffer most. The post harvest losses to fruits would depend upon the moisture content of fruits (Frazier & Westhoff, 1989).

To minimize the losses the contact between micro organisms and our foods (to prevent contamination) and also

elimination of microorganisms from our foods or adjusting condition of storage to prevent their growth (Frazier and Westhoff, 1989) are essential. If the microorganisms involved in damage are pathogenic their association with our food supply is hazardous from a public health point of view. Many of our foods support the growth of pathogenic microorganisms or serve as a vector of them. Emphasis should be given to prevent their entrance and growth in our foods or eliminate them by processing (Frazier & Westhoff, 1989).

The microorganisms not only spoil the fruits in appearances but reduces the quality of fruits and the nutrient value. Some of the microorganisms causing perishables diseases of fruits and vegetables cause the ailment of human being and animals (Dasgupta & Mandal, 1989).

Mandal and Dasgupta (1983) isolated at least 17 fungal pathogens from perishables in West Bengal, which are known to cause diseases in man and animals. Besides, there are bacteria obtained from the decomposing perishables which at the same time may cause disease in animals (Mandal & Dasgupta, 1983). Alcaligens faecalis, Enterobacter spp. etc. constitute natural enteric flora in the warm blooded animals and may turn pathogenic (Frazier & Westhoff, 1989).

There are large number factors which affect post harvest losses of perishables fruit and vegetables (Bhargava 1962;

Eckert, 1978; Liu & Ma, 1983). However, the magnitude of post harvest loss is influenced by initial quality of the produce, environmental, operational, physiological changes during storage and genetical factor.

Amongst the physical factors, temperature and relative humidity are known to play a crucial role in decaying process of fresh products (Pantastico, 1975; Harvey, 1978) by influencing growth of the pathogens as well as in pathogenesis. The microbial attack to different crops becomes very slow at temperatures below 5°C (Pathak, 1972; Harvey, 1978). The optimum temperatures near 0°C are suited for many fruits and vegetables viz. apple, orange, carrot, grape (Eckert, 1978). Chilling injury is noticed in tropical fruits of mango, banana, lemon, tomato, grape fruit, pineapple when stored at 10-15°C and below 12°C in grape fruit which reduces host resistance against Alternaria stem end rot (Schiffman-Nadel et al. 1975; Eckert, 1978). Geotrichum and Erwinia sp. are inhibited at 0°C but can rapidly cause disease when the produce is brought back to high temperatures after storage at 0°C. Botrytis, Penicillium, Selerotinia, Centrospora, Cladosporium etc. grow slowly at near freezing temperatures. Rhizopus stolonifer neither grows nor their spores germinate below 7.5°C.

Relative humidity near saturation results in heavy losses except at temperature near 0°C. RH equilibrium below 90% doesn't permit microorganisms to grow on crop surface (Singh et al., 1983).

Deleterious effects of controlled atmosphere on enhancing disease incidence have also been reported bacterial soft rot of immature tomato is reported in atmosphere of carbon dioxide (5%) + oxygen (3%) (Pearson & Hall, 1975). Ethylene, a ripening inducer, enhance rotting in Alternaria rot of tomatoes; stem end rot and anthracnose of citrus (Eckert, 1978).

Proper packaging and handling can reduce injuries and moisture loss, prevent recontamination and maintain the desirable storage atmosphere. Plastic films of low permeability to water vapour are widely used for packaging consumer units of fresh fruits and vegetables in developed countries (Grierson, 1969; Eckert, 1975; Harvey, 1978) which reduces the spoilage of fruits. Singh and Kainsa (1983) observed that bamboo basket for grapes reduces their spoilage than wooden box.

The fruits and vegetables during post harvest period are attacked by several pathogens at a time (DGM). The association have been identified as belong to any one of the following categories :

1. Non interfering coincidence
2. Predominance of one
3. Unilateral aggravation
4. Unilateral retardation
5. Mutual aggravation
6. Synergism
7. Occupational privilege of the pioneer settler

8. Copathogenicity

9. Antagonism

Fruits undergo tremendous biochemical changes during ripening (Thind et al.1977) but also during infection. One of the most obvious of the changes that occur during fruit ripening is the conversion of insoluble carbohydrate to soluble sugars. In a number of fungal diseases of plants the general resistance to colonization has been related to the sugar content of the tissue (Horsfall & Dimond, 1957). In the same year Horsfall and Dimond (1957) reported that diseases in which susceptibility was favoured by high sugar levels were referred to as high sugar disease and the converse as low sugar diseases. If increasing sugar levels responsible for the susceptibility to rotting in fruits, then such post harvest disease would be high-sugar diseases.

Considerable studies have been made on the association of two or more microorganisms on fruits in causing damage (Mandal & Dasgupta, 1982). The phenomenon of antagonism has also been used for adopting biological control. But information about the chemical changes brought out inside the fruits as a result of infection with two or more pathogens specially the antagonists is very scanty. Most of the studies deal with changes brought out due to infection with single pathogens. Therefore, in the present studies an attempt will be made to determine the biochemical changes in the fruits as a result of infection of fruits with 2 or more pathogens during post harvest operation.

REVIEW

OF

LITERATURE

The surfaces of healthy fruits include the natural flora plus contaminating microorganisms from soil and water. In addition, some fruits contain plant pathogens or saprophytic spoilage organisms which may grow subsequent to harvesting. A few microorganisms are present in the interior of occasional healthy fruits (Pantastico, 1975).

The microorganisms on the surfaces of freshly harvested fruits and vegetables include not only those of the normal surface flora but also those from soil and water. Pseudomonas, Alcaligenes, Bacillus, Chromobacterium, Enterobacter, Flavobacterium, Lactobacillus, Leuconostoc, Micrococcus, Sarcina, Serratia, Staphylococcus, in addition to genera causing plant pathogens make normal surface bacterial flora. In case the surfaces is moist or the outer surface has been damaged, growth of some of these microorganisms may take place between harvesting and processing of the vegetables. Some of the important non-pathogenic diseases include brown heart of apples and pears, black heeart of potatoes and red heart of cabbage (Tomkins, 1951; Vaughn, 1963).

Large number of bacteria have been reported to cause rots of fruits and vegetables. Some of the details are given in table 1 and 2.

Table No. 1 : Bacteria that cause soft rot of vegetables

Bacterium	**Produce affected**	Author
Erwinia carotovora sub sp. atroseptica	Most vegetables particularly potatoes and some fruits.	Perombelon & Kelman (1980)
E. carotovora sub sp. carotovora	Most vegetables & some fruits.	"
Pseudomonas marginalis	Many vegetables	Doudoroff & Pelleroni (1974)
P. viridiflava	Beans	"
P. cepacia	Onion	"
P. gladioli pv. allicola	Onion	"
B. polymyxa	Potatto, Pepper	Brocklehurst & Lund (1982)
Bacillus subtilis	Potato	Gibson & Gordon (1974)
Clostridium puniceum	Potato	Lund et al. (1981)
Low temperature Clostridium	Potato	Brocklehurst & Lund (1982)

Table No. 2 : Bacteria that cause diseases of fruits.

Fruit	Disease	Pathogen	Reference
Grape	Bacterial blight	*Xanthomonas ampeline*	Winkler *et al.* (1974)
	Crown-gall	*Agrobacterium tumefaciens*	Teviotdale & Schroth (1981)
	Other diseases	*Pseudomonas viticola*	Hedge & Kulkarni (1985)
		Xanthomonas vitis-vinifera	Nayudu (1972)
Pineapple	Fruit collapse	*Eriwinia chrysanthemi*	Lim & Lowing (1983)
	Heart rot	*E. chrysanthemi*	Lim (1974); Perombelon & Kelman (1980)
	Brown rot	*Pseudomonas ananas*	Serrano (1928)
		& or	
		E. ananas	Young *et al.* (1978)
	Pink diseases	*Gluconobacter Acetobacter Enterobacter*	Rohrbach & Pfeiffer (1976)
Pomegranate	Bacterial blight	*X. campestris*	Ramesh & Ramkishan

E. carotovora sub sp. *betavasculorum* caused soft rot and vascular necrosis of sugar beet (Thomson & Schroth 1981) in addition to several diseases of fruits and vegetables. According to Chupp and Sherf (1960) the *E. carotovora* group of bacteria are probably the major single cause of microbial spoilage of vegetables.

Post-harvest soft rots are also caused by E. carotovora (Ramsey et al., 1959; Smith et al., 1966; Smith and Wilson, 1978). It is responsible for the cause of blackleg of potatoes (Perombelon & Kelman, 1980). Cauliflower and cabbage have also been found to be spoiled by the group of organisms (Keller and Knosel, 1980). E. carotovora and E. chrysanthemi are reported to cause core rot found in carrots (Towner and Beraha, 1976). Victoria & Granada (1983) pointed out that E. chrysanthemi caused losses of tomatoes upto 60%. E. amylovora caused a severe outbreak of fire blight affecting greater than 43000 apple and pear annually (Phocas, 1986).

Strains of Pseudomonas marginalis are capable of causing post harvest spoilage of many vegetables e.g. celery (Harrison and Barlow, 1904), chicory (Friedman, 1951), Lettuce (Dowson, 1941; Ceponis, 1970; Beraha and Kwolek, 1975), cabbage (Bobbin and Geeson, 1977) and lettuce (Hall et al. 1971). P. viridiflava was first described as the cause of reddish-brown necrotic lesions on a market sample of green bean pods (Burkholder, 1930). It also caused soft rot of cabbage and lettuce leaves (Wilkie et al. 1973). P. chichori caused post-harvest spoilage of cabbage (Smith and Ramsey, 1956) and lettuce (Ceponis, 1970; Grogan et al., 1977). The other species like P. aeruginosa caused post harvest spoilage of vegetables. An outbreak of spoilage of onion bulbs in Australia in 1974 was attributed to P. aeruginosa. In a shipment of tomatoes from Florida 17% of the 'decay bacteria' isolated were identified as P. aeruginosa, 17% were P. marginalis

and 66% Erwinia sp. (Bartz, 1980).

There are a few reports that species of Bacillus are able to cause spoilage of vegetables. The Bacillus failed to attack carrot or turnip roots, cabbage heads and other plant parts. B. subtilis group was isolated as the cause of soft rot in tomatoes and caused extensive rot at 36-40°C (Madhok & Fazal, 1943).

Clostridium sp. were isolated from potatoes that showed extensive soft rot (Campos et al., 1982; Lund, 1982b). These are usually present alongwith E. carotovora, but in some condition act as primary cause of rots (Perombelon & Kelman, 1980). Several morphological types of Clostridium have been isolated from rotting potatoes (Lund et al., 1981; Perombelon & Kelman, 1979; Campos et al., 1982).

Nedumaran and Vidhyasekaran (1981) studied the production of pectic enzymes by Corynebacterium michiganens in tomato fruits. Tomato lines with varying degrees of resistance to bacterial wilt (P. solanacearum) have been reported by Tikoo et al. (1984). Tomato pith necrosis caused by P. viridiflava (Al vizatos, 1986). Calzolari (1986) discussed various bacterial diseases of tomato i.e. Bacterial spot by Xanthomonas campestris, Bacterial speck by Pseudomonas syringae, necrosis of the medulla by P. corrugata and Bacterial canker due to Clavibacter michiganense and Corynebacterium michiganense (Kurozawa, 1984). Pseudomonas corrugata found to be causative agent of tomato pith necrosis

(Olsson, 1986). According to Sasaki & Umekawa (1986) Bacterial canker (Corynebacterium michiganense, Clavibacter michiganensis) occurred when the population of pathogenic bacteria reached 10^6 cells/gm tomato, but didn't occur at lower level. Xanthomonas sp. & Pseudomonas sp. isolated in mixed culture from tomato (Gitaitis et al., 1987).

FUNGAL SPOILAGE OF FRUITS AND VEGETABLES :

When apples and pears ripen they become susceptible to attack by a variety of fungi to which they were resistant during their period of development on the tree (Edney & chambers 1981). Considerable damage due to rotting caused mainly by Gloeosporium spp. and Monilia fructigena on apples has been reported (Preece, 1967). Swinburne (1970) reported average losses of apples as 15% in refrigerated gas stores and 3% in air (barn) stores. The main species responsible for rotting included Nectaria galligena and Penicillium expansum which accounted for more than half of all the rots examined. A variety of fungal forms including Gloeosporium, M. fructigena, Penicillium, Botrytis cineria, N. galligena, Aspergillus, Rhizopus, Trichothecium and Phoma have been known to cause rotting of apples as well as pear fruits. Gloeosporium, Monilia and Penicillium were found to be main causative agent of rotting (Babovic et al., 1980; Vyas et al., 1976). Cryptosporiopsis and Phylactaena spp. have been reported to induce lenticel rot during post harvest period (Thind et al., 1977). Both apple and pear have been shown to be susceptible

to Sclerotium rolfsii (Sumbali and Mehrotra , 1980) and Monili.. a taxa (Kaul & Munjal, 1981).

The most important post harvest pathogen of soft fruits is Botrytis cinerea (Dennis & Mountford, 1975; Davis & Dennis, 1977; Mason & Dennis, 1978). Other fungi such as Mucor and Rhizopus spp mainly infect strawberries and to lesser extent on respberries (Dennis & Davis, 1977; Mason & Dennis, 1978) and Cladosporium spp. infect the surface responsible for spoilage of strawberries are Colletotrichum fragariae (Howard, 1972), Dandrophoma obscurans, Pestalotia longischila, Alternaria tenuissima (Howard and Albregts, 1974), Phytophthora nicotianae (Matsuzaki et al., 1981), P. citrophthora (Kao and Leu, 1981).

Species of Rhizopus, Aspergillus, Colletotrichum, Botryodiplodia, Pestalotia, Phomopsis and Diplodia have been reported to cause rotting of fruits of mango during marketing, transit and storage (Verma & Kamal, 1951; Thakur, 1972; Laxaminarayana & Reddy, 1975 ; Thakur & Chenulu, 1970) reported that R. arrhizus alone was responsible for decay of 6.3% of mango fruits in Delhi market. Species of Aspergillus, Botryodiplodia, Pestalotia, Phoma, Phomopsis and Rhizopus cause rotting of fruits, Gloeosporium spp. not only caused anthracnose but rotting of fruits as well (Dhingra and Mehrotra, 1980;. Saxena & Saksena, 1983). Besides these Curvularia, Monilia, Fusarium and Aspergillus, Phytophthora, Macrophoma, Pestalotia, Dichotomyces and Physalospora have also been reported to attack 15-20% fruit during

storage in Agra (Gupta & Madaan, 1970). According to Ooke (1981) R. slolonifer is also serious pathogen of post harvest insears. Colletotrichum musae, Fusarium roseum, Verticillium theobromae and Ceratocystis paradoxa. Gloeosporium musarum caused post harvest disease as well as anthracnose (Sudhir Chandra, 1986) of banana Trichothecium roseum (Srivastava & Tandon, 1971). F. moniliforms (Khanna & Chandra, 1976) also attacked fruits in storage. Botryodiplodia (Lantican & Quimo, 1978), F. monliforme and F. roseum (Khanna & Chandra, 1976), A. terrus (Sharma & Khan, 1981) have been known to cause post harvest losses of fruits. According to Sattar Haithami (1986) Macrophomina phaseoli (Charcoal rot) is responsible for banana fruit spotting.

Papaya fruits are damaged most by Botryodiplodia, Phytophthora, Trichothecium and Cladosporium. Colletotrichum spp. and Gloeosporium spp. have been reported to cause rotting as well as anthracnose during storage (Grewal, 1954). Prasad and Verma (1976) while making a survey of the diseases of fruits during storage found that Alternaria, Chaetomium, Fusarium and Rhizopus were responsible for the spoilage. Other pathogens reported as post harvest cause of loss are Verticillium theobromae (Srivastava & Tandon, 1971); Macrophomina phaseolina (Kapur & Chohan, 1974), F. solani (Quimio & Quimo, 1977), Colletotrichum capsici (Lal et al. (1980). Trichothecium roseum (Saxena & Jain, 1981) & Chaetosphaeropsis truncata (Rai and Bihari Lal, 1984) Cladosporium harbarum, Botrytis (Jainini, 1923); Botrytis cinerea, Penicillium digitatum, P. italicum, A. citri, Cladosporium spp.

(Adam, 1923), Diplodia natalensis (Abbot, 1931), caused considerable decay of citrus fruits. Besides these other fruit rot fungi of citrus which cause damage include B. theobromae, F. moniliformae, F. fumigatus, Colletotrichum gloeosporbides, Aspergillus niger (Srivastava & Tandon, 1969). Curvularia tuberculata, C. lunata, A. tenuis, R. nigricans and R. stolonifer (Garcha et al. 1980), Phytophthora citrophthora, P. parasitica (Feld & Mange 1979). Geotrichum candidum (Kamal et al., 1978), Penicillium digitatum, P. italicum, (Bai, 1979).

Phytophthora spp are also found associated with post harvest rotting of citrus fruits (Ullasa, 1986).

Microbial spoilage is generally brought out by species of Botrytis, Cladosporium, Gloeosporium, Alternaria, Stemphylium and Aspergillus (Mac clellan & Hewitt, 1973; Prakash et al., 1976; Sudhir Chandra, 1986).

Geotrichum candidum, Aspergillus spp. Collectotrichum spp. and Pestalotia spp. have been reported to cause rotting of fruits of lithi (Jamaluddin et al., 1975)

Phomopsis versoniana caused a severe fruit rot of pomegranate (Mehta et al. 1975) Gloeosporium spp. Fusarium spp. Penicillium spp. and Aspergillus spp. other well known organisms

causing fruit rots (Srivastava & Tandon, 1971; Philip, 1981) of pomegranate.

Only a few fungal spp. such as Ceratocystis paradoxa and R. stolonifer have been responsible for decay of pineapple.

Tandon (1967) reported Botryodiplodia theobromae, Pestalotia sapotae and Hendersonula torulcidea as the cause of decay of sapota fruits.

Peaches are attacked by wound pathogens e.g. A. niger and B. theobraomae (Sumbali & Mehrotra, 1980; Bhargava et al., 1979) which cause tremendous losses.

Botrytis cinerea is the major cause of post harvest fruit rotting of tomatoes (Chastagner and Ogawa, 1979; Dennis and Davis, 1980). For outdoor grown fruits Alternaria spp. are mainly causative pathogens (Bartz, 1972; Pearson & Hall, 1975; Dennis & Davis, 1980). Other fruit rotting fungi are Stemphylium spp. Fusarium spp. Cladosporium spp. and R. stolonifer, Pythium spp. (Pearson & Hall, 1974). Phoma destructiva, Nigrospora oryzae, Phytophthora, P. nicotianae, Myrothecium carmichaeli (Kogan, 1979; Tandon & Singh, 1981) and Geotrichum candidum, Rhizoctonia solani, Botryodiplodia theobromae (Bartz, 1980). According to Singh et al. (1983) post harvest decay of ripe tomato fruits is caused by Cladosporium oxysporum.

Phocas (1986) reported the presence of Fulvia fulva on tomato. Phoma andina was found to be new pathogen of

tomato (Loerakkar et al., 1986). This pathogen caused necrosis followed by mummification of fruit.

The causative pathogen of brinjal are Phytophthora spp., Rhizopus spp., Phoma exigua, Fusarium moniliforme, F. solani, Solanum melongena, Alternaria, Sclerotium rolfsii and Phomopsis spp.(Chowdhry & Hasija, 1981; Ali & Shukla, 1981; Datar, 1981).

Sharma (1981) isolated P. digitatum from Aegle marmelos; Cladosporium herbarum from Avverhoa carambola; Ulocladium chartarum from Carica papaya, Alternaria eribotryae from Eribotrya japonica, A. tenuissima and Myrothecium roridum from Memordica charantia, A. terrans and Cylindrocarpon radicicola from Musa paradisiaca, A. alternata, C. cladosporiodes and P. expansum from Physalis peruviana; Curvularia pallescens from Pyrus communis; A. asculeatus from Spondias mangifera and Penicillium chrysogenum from Vitis vinifera are found to be pathogens.

The perishables undergo drastic changes in biochemical status of fruits and vegetables as a result of infection. Considerable studies have been made both in the favour on the changes in carbohydrates, proteins, protein-bound and free amino acid due to pathogens on large number of fruits and vegetables both in tropics and temperate areas (Dasgupta & Mandal, , 1989). It is difficult to review the entire literature. Therefore, some of important studies carried out on changes in biochemical status of fruits and vegetables like tomato, banana, brinjal, guava, have been summarised in the tables.

CARBOHYDRATE :

Drastic changes in sugar contents was observed in pineapple, banana, mango, sapota and musambi fruits infected with Botryodiplodia theobromae (Bhargava, 1962); banana with Fusarium oxysporum (Chandra & Tandon, 1963); cucumber by Pythium aphanidermatum (McCombs & Winstead, 1964).

Banana infected with B. theobromae showed decrease in fructose and glucose content (Williamson & Tandon, 1965).

Oligosaccharides were not detected in fruits infected with Pestalotia sapota and B. theobromae on sapota, Gloeosporium psidii and Phoma psidii on guava (Tandon, 1967). However, oligosaccharides were detected in mango fruits infected by Colletotrichum gloeosporioides (Ghosh et al., 1965a), while in

musambi fruits infected with B. theobromae the oligosaccharide developed on the 4th day after infection (Srivastava & Tandon,1966b)

A significant decrease in sucrose, glucose and fructose was found in guava fruits infected with Machrophomina allahabadensis (Kapoor & Tandon, 1967); pomegranate, guava, musambi by A. niger (Singh, 1968); banana and papaya with R. stolonifer; tomato with Phoma destructiva (Aulakh et al. 1970b); chilli fruits with Choanephora cucurbitarum (Chahal & Grover, 1972); banana with Cochliobolus spicifer & Alternaria alternata (Prasad, 1974), Papaya with Phomopsis caricae (Dhingra & Khare, 1975); tomato with A. solani (Mehta et al., 1975b) and musambi fruits with B. theobromae (Ali, 1976). Such changes were not detected in healthy vegetables and fruits.

Qualitative and quantitative difference in the sugar contents in healthy and diseased fruits have been worked out by many workers (Chahal and Grover, 1972; Ghosh et al., 1964; Kapoor & Tandon, 1971; Singh & Sinha, 1983, 1984). The decrease in carbohydrate in the fruit tissue has been attributed to

i) breakdown of carbohydrates by the enzymes produced by the pathogens.

ii) increased respiration of the infected tissue

iii) utilization of carbohydrates as substrate for the production of metabolites.

Table No. 3 : CHANGES IN CARBOHYDRATE CONTENT OF PERISHABLES DURING PATHOGENESIS

Host	Organism	Carbohydrates	Changes noted	References
Banana	Alternaria alternata	Sucrose, glucose, fructose	Contents decreased	Prasad (1974)
	Botryodiplodia theobromae		Content exhausted	Williamson & Tandon (1965)
	Chaetosphaeropsis sp.		Glucose, fructose exhausted, sucrose considerably decreased	Rai (1982)
	Fusarium oxysporum		Sucrose considerably decreased; other sugars remain same; new sugars developed	Chandra and Tandon (1963)
	Gloeosporium musarum		Sucrose exhausted, other sugars gradually decreased	Tandon (1970)
	Helminthosporium specifierum		Sugars considerably decreased	Prasad (1974)

Guava	Aspergillus flavus	Monosaccharides sugars	Both reducing and non reducing sugars decreased	Singh and Sinha (1983)
	A. niger	glucose, sucrose, fructose	Quantity decreased with time	Singh (1968)
	A. parasiticus	Sugars	Both reducing and non reducing sugars decreased	Singh and Sinha (1983)
	Botryodiplodia theobromae	Sucrose, glucose, fructose, monosaccharides	Hexose sugars considerably reduced	Tandon (1970), Srivastava & Tandon (1969)
	"	"	Fructose consumed earlier than glucose and sucrose	Ghosh et al. (1964)
	Gloeosporium psidii	Glucose, sucrose, fructose	Sugars slowly utilised	Ghosh et al. (1964)
	Macrophoma allahabadensis	Sucrose, glucose d-fructose	Content gradually decreased; an oligosaccharide newly formed	Kapoor and Tandon (1969)
	Pestalotia psidii	Sucrose, glucose, fructose	Sucrose exhausted by 6 days; glucose consumed earlier than sucrose	Ghosh et al. (1964)

	Macrophoma allahabadensis	Sucrose, glucose d-fructose	Sucrose completely utilised	Kapoor and Tandon (1971)
	Phoma psidii	Sucrose, glucose, fructose	Sucrose exhausted by 6 days; glucose consumed earlier than fructose	Ghosh et al. (1964)
	Phomopsis destructum	"	Contents gradually decreased	Rao and Agarwal (1975) Acc. to Dasgupta & Mandal (1989)
	Phytophthora nicotianae	"	All sugars considerably reduced	Mathur et al. (1980) Acc. to Dasgupta & Mandal (1989)
Tomato	Alternaria alternata	"	Glucose and maltose exhausted; others decreased	Mehta et al. (1975)
	A. solani	Glucose, fructose maltose, sucrose	Glucose and maltose exhausted; sucrose and fructose decreased	Mehta et al. (1975)
	Colletotrichum sp.	Glucose, fructose, maltose	Maltose exhausted; fructose and glucose considerably decreased	Tandon et al. (1974)

	Cylindrocladium scoparium	Maltose, glucose, sucrose	Content considerably decreased	Tandon et al. (1974)
	Drechslera australiense	Maltose, glucose, sucrose, fructose, oligosaccharides	Content considerably decreased	Kapoor and Tandon (1970)
	Myrothecium roridum	Fructose, glucose, maltose	Contents considerably reduced	Tandon et al. (1974)
	Nigrospora oryzae	Fructose, maltose, glucose, sucrose	Sucrose and maltose exhausted; glucose and fructose considerably decreased	Reddy et al. (1984)
	Phoma exigua	Sucrose, glucose, fructose, maltose	Except fructose others exhausted	Reddy et al. (1984)
	Rhizoctonia solani		Contents exhausted within 8 days	Reddy et al. (1984)
Brinjal	Alternaria alternata	Sucrose, glucose, fructose, other oligosaccharides	Fructose exhausted; sucrose and glucose considerably declined; oligosaccharides newly formed	Prasad and Poddar (1977)

Helminthosporum	glucose, fructose,	Fructose exhausted;	Prasad and Poddar
speciferum	sucrose	glucose considerably	(1977)
		decreased; sucrose	
		decreased but later	
		on increased; other	
		oligosaccharides newly	
		formed.	

According to Sharma (1981), qualitative analysis of the healthy and diseased fruits and vegetables juices for the presence of different carbohydrates showed that nearly all the healthy fruits and vegetables contained glucose, fructose and sucrose but for banana where starch was present and grapes where only glucose was detected. However, in some infected fruits and vegetables these sugars disappeared simultaneously with the appearance of an unknown sugar when infected with P. digitatum, U. chartarum, A. eribotryae, A. terreus, C. cladosporioides, P. expansum and C. pallescens infected fruits. Total sugar content was lower in Aspergillus sp. infected tissues (Verma, 1991).

During pathogenesis there is an increase in the loss of complex carbohydrate and initial decrease in single sugars follows by decrease (Table No. 3).

PROTEIN AND AMINO ACIDS :

Infection of the fruits causes many changes in the amino acid contents (Kapoor & Tandon, 1969a, 1970, 1971; Chahal & Grover, 1972). It is considered that absence or decrease in the concentration of amino acids in infected fruits may possibly be attributed to their preferential utilization by the fungus or to their degradation by enzymes or simultaneous utilization in synthesis of protein.

In cucumber fruits infected with Pythium aphanidermatum (McCombs & Winstead, 1964); blue berry with Glomerella cingulata

(Strech & Cappellini, 1965); guava fruits with B. theo bromae, Phoma psidii; banana fruits with Gloeosporium musarum (Tandon, 1967). Pomegranate, mango and guava fruits infected with Aspergillus niger (Singh, 1968); Tomato with Drechslera australiensa (Kapoor & Tandon, 1969a) and citrus fruits with B. theobromae (Srivastava, 1966a); chilli fruits with Choanephora cucurbitarum (Chahal & Grover, 1972), and tomato fruits with Alternaria solani (Mehta et al., 1975 a & b) decrease in amino acids contents have been reported.

Giorbelidze (1987) worked on effect of the fungus Macrophoma mantazziana on the composition and content of free amino acids in Lemon. When the susceptible variety Gruizinskii and the relatively resistant Meyer and Dioskuriya were artificially infected with the fungus, content of free amino acid fall in all varieties, having been highest in healthy tissues of the resistant varieties. At the early stage of infection, the quantitative composition of amino acids fell in all varieties, but at the more advanced infection stage the range (composition) of amino acids increased even beyond that in healthy tissue in the susceptible variety but not in the resistant.

According to Sharaf et al. (1989) 14 amino acids can be detected in apricot and 12 in mango and percentage of each varied according to the degree of ripening.

Both free and protein-bound fractions increase during pathogenesis, probably due to proteolysis of the host-protein.

Table No. 4 : CHANGES IN PROTEIN, PROTEIN-BOUND AND FREE AMINO-ACIDS IN PERISHABLES DURING PATHOGENESIS

Host	Organism	Content	Results	References
Banana	Alternaria alternata	Free amino acids	Arginine & glutamic acid content remain same and some unidentifiable amino acids formed	Prasad (1981) Acc. to Dasgupta & Mandal (1989)
	Botryodiplodia theobromae	Free amino acids	Serine, glycine and valine increased; others remain same	Tandon (1967)
	Chaetosphaeropsis sp.	Free amino acids	Contents reduced	Rai (1982)
	Gloeosporium musarum	Free amino acids	Serine and glycine increased; others remain same	Tandon (1967)
	Helminthosporium speciferum	Free amino acids	Unidentifiable amino acids formed; others remain same	Prasad (1981) Acc. to Dasgupta & Mandal (1989)
	Rhizopus stolonifer	Free amino acids	Serine, glycine increased; others remain same	Tandon (1967)

Guava	Aspergillus flavus	Protein	Reduced by 45.3%	Singh and Sinha (1983)
	A. niger	Free amino acids	Y-amino-butyric acid, proline, asparagine exhausted, leucine/ isoleucine, glutamic acid reduced; ∝-alanine, arginine, aspartic acid, serine/ glycine enhanced; thronine newly formed; valine unchanged	Singh (1968)
	A. parasiticus	Protein	Content reduced by 17.6%	Singh and Sinha (1983)
	Botryodiplodia theobromae	Free amino acids	Aspartic acid considerably reduced; asparagine, ∝-alanine, arginine, Y-aminobutyric acid remain same or decreased, serine, glycine increased	Srivastava and Tandon (1969)
	Curvularia tuberculata	Free and protein-bound amino acids	Changes noted	Kapoor (1983) Acc. to Dasgupta & Mandal (1989)

Gloeosporium psidii	Free amino acids	Serine, glycine, Y-amino-butyric acid considerably reduced; aspartic acid, glutamic acid, asparagine, ∝-alanine, arginine remain same	Tandon (1967)
Macrophoma allahabadensis	Free amino acids	Leucine, isoleucine, valine, proline, threonine exhausted; ∝-alanine, glutamic acid, arginine, glutamic acid, arginine, aspartic acid, asparagine reduced, serine/glycine increased	Kapoor (1968); Kapoor and Tandon (1971)
Pestalotia psidii		Serine, glycine, Y-amino-butyric acid considerably reduced; others remain same; valine newly formed	Tandon (1967)
Phoma psidii	Free amino acids	Y-aminobutyric acid considerably reduced;	Tandon (1967)

			others remain same; serine glycine increased	
	Phytophthora nicotianae	Free amino acids	Content reduced	Mathur et al (1980) Acc. to Dasgupta & Mandal (1989)
Tomato	Alternaria alternata	Amino acids	Either ammonia released or asparagine accumulated	Abdel-Rehim et al. (1974).
	A. solani	Free amino acids	Cystine, tyrosine, valine, isoleucine/lecucine, asparagine newly formed; methionine, asparagine increased and degraded; others remain same	Mehta et al. (1975)
	Collectotrichum state of Glomerella cingulata	Free amino acids	Threonine, arginine, asparagine, cysteic acid exhausted; serine and glycine considerably reduced; aspartic acid, glutamine, histidine remain same; leucine, valine, Y-aminobutyric acid,	Tandon et al. (1974)

		∝-alanine, glutamic acid increased; proline newly formed.	
Cylindrocladium scoparium	Free amino acids	Threonine, histidine, lysine, cysteic acid exhausted, serine, glycine, asparagine slightly reduced, arginine, aspartic acid remain same; increased in leucine, isoleucine, valine Y-aminobutyric acid, ∝-alanine, glutamic acid, glutamine increased; proline newly formed.	Tandon et al. (1974)
Drechslera australiense	Free amino acids	Arginine exhausted; valine, threonine, tyrosine, glutamine newly produced; others remain same.	Kapoor and Tandon (1969)
Geotrichum candidum	Free amino acids	Either ammonia released or asparagine accumulated	Abdel-Rehim et al. (1974)

Myrothecium roridum	Free amino acids	Cysteic acid exhausted; threonine, serine, glycine, asparagine decreased, tyrosine, proline, Y-aminobutyric acid, aspartic acid remain same; isoleucine/leucine, valine, ∝-alanine, glutamic acid, arginine, histidine, lysine increased	Tandon et al. (1974)
Nigrospora oryzae	Free amino acids	Leucine, isoleucine, β-alanine, glutamic acid considerably reduced; glycine, serine enhanced; aspartic acid newly formed.	Reddy et al. (1984)
Phoma exi	;ids	∝-alanine, glutamic acid reduced; others exhausted; aspartic acid newly formed	Reddy et al. (1984)

	Rhizoctonia solani	Free amino acids	∝-Asparagine, histidine, lysine exhausted; others reduced	Reddy et al. (1984)
Brinjal	Alternaria alternata	Free amino acids	Valine and leucine completely utilised; threonine, asparagine, tyrosine and cystein partially used	Prasad and Poddar (1977)
	Helminthosporium speciferum	Free amino acids	Cystein, glutamic acid, asparagine, leucine, exhausted; valine, tyrosine considerably reduced; arginine remains same; threonine increased.	Prasad and Poddar (1977)

There are evidence to show reduction or disappearance of some amino acid like valine in infected tissue (Table No. 4). In some cases enhanced synthesis of protein (in sweet potato infection with Ceratocytis fimbriata; in strawberry infection with R. stolonifer and Mucor piriformes (Thompson & Eribo, 1984). Increased protein content of affected tissues due to the association of fungal mycelium with host tissues. The total free amino acid content of infected tissues was reduced by infection of Aspergillus sp. (Verma et al., 1991).

ORGANIC ACIDS :

Organic acids are important constituents of the fruits Drastic changes were found in tartaric, fumaric succinic, oxalic, malic acids which are constituents of many fruits and vegetables. Srivastava & Tandon (1966) observed disappearance of organic acids in guava, mango, musambi and sapota fruits infected with B. theobromae. Changes in organic acid content has been reported in chilli infected with R. stolonifer (Tandon et al., 1974); and tomato fruits infected with Alternaria solani and A. tenuis (Mehta et al., 1975 a & b) and in peach fruit during infection by A. flavus (Jamaluddin, 1979).

During pathogenesis, the concentration of some organic acids increase while in others there is a decrease (Dasgupta & Mandal, 1989) citric, fumaric, malic, malonic, oxalic, succinic acid decrease probably because these are consumed during respiration. However in early phases of pathogenesis, there could be an increase (McElroy, 1961).

Table No. 5 : CHANGES IN ORGANIC ACIDS IN PERISHABLES DURING PATHOGENESIS

Host	Organisms	Results	References
Banana	A. alternata	Great changes noticed	Prasad (1981)
	Helminthosporium specifernm	Changes noted	Prasad (1981) Acc. to Dasgupta & Mandal (1989)
Guava	A. niger	Fumaric acid and an unidentified organic acid newly formed; others increased	Singh (1968)
	Botryodiplodia theobromae	No change in citric acid; malic acid reduced; tartaric acid, fumaric acid and succinic acid exhausted	Srivastava & Tandon (1966)
	Curvularia tuberculata	Contents changed	Kapoor (1983)
	Macrophoma allahabadensis	Marked changes noted	Kapoor and Tandon (1971)
	Phomopsis psidii	Total organic acids initially increased, followed by a gradual decrease after 10 days	Arya (1982) Acc. to Dasgupta & Mandal (1989)

Tomato	A. alternata	Succinic acid newly formed; no difference in content of others	Mehta et al. (1975)
	Colletotrichum sp.	Malic acid, considerably formed, tartaric acid remains same, citric acid much decreased and oxalic acid exhausted	Tandon et al. (1974)
	Cylindrocladium scoparium	Malic and fumaric acids newly formed; tartaric acid remains same; citric acid considerably reduced; oxalic acid exhausted	Tandon et al. (1974)
	Geotrichum candidum	Contents changed	Jamaluddin et al. (1978)
	Myrothecium roridum	Fumaric acid newly formed; malic acid increased; tartaric acid reduced; citric acid and oxalic acids exhausted.	Tandon et al. (1974)
Brinjal	A. alternata	Malic acid increased; succinic acid remains same and fumaric acid exhausted	Prasad and Poddar (1977)
	Helminthosporium speciferum	Succinic, malic and fumaric acids exhausted	Prasad and Poddar (1977)

Twelve fungi	Contents of succinic, malic and formic acids greatly changed.	Shreemali (1983) Acc. to Dasgupta & Mandal (1989)

In tomato infected by D. australience, succinic acid is produced by host which is reduced by the fungus (Kapoor & Tandon, 1969; Tandon, 1970). This fungus is not able to produce the acid in vitro (Table No. 5).

ASCORBIC ACID :

Ghosh et al. (1965); Srivastava and Tandon (1966); Kapoor and Tandon (1969b); Tandon and Jamaluddin (1973); Thind et al. (1977); Kapoor (1982); Prasad and Bilgrami (1979) have reported that infection by pathogen induced marked changes in the ascorbic acid (vitamin C) content in different fruits. According to Tandon (1970) the loss of vitamin C during pathogenesis may be due to production of suitable ascorbic acid degenerating enzymes either by the pathogen alone or by the host-pathogen complex. Increased respiration in diseased tissue may also be responsible for a decline of vitamin.

Reduction in ascorbic acid contents has been observed in guava infected with Phoma psidii (Ghosh et al., 1965b), mango fruits with Aspergillus niger and papaya fruits with B. theobromae (Ghosh et al., 1966).

Rate of decrease of ascorbic acid was faster in mango fruits infected with B. theobromae only 10.8% ascorbic acid remained in apple coloured variety of guava while none was left in safeda variety, both infected with B. theobromae and no

Table No. 6 : REDUCTION IN ASCORBIC ACID CONTENT OF PERISHABLES DURING PATHOGENESIS

Host	Organism	Initial Content mg/100g tissue	Period of incubation (in days)	Observations (% loss)	References
Banana	A. alternata	45.62	15	62.9	Prasad and Prasad (1977)
	Helminthosporium				Acc. to Dasgupta & Mandal (19
	speciferum	45.6	10	70.8	Prasad (1977)
Guava	A. flavus	280	10	26.0	Singh and Sinha (1983)
	A. niger	360	12	96.0	Singh and Tandon (1971)
	A. parasiticus	360	10	Considerably depleted	Singh and Sinha (1983)
	Botryodiplodia theobromae	305.2	12	89.0	Srivastava and Tandon (1966)
	Curvularia tuberculate	34.6	10	81.4	Kapoor (1983)
					Acc. to Dasgupta & Mandal (1
	Gloeosporium psidii	305.2	12	90.0	Ghosh et al. (1966)

	Macrophoma allahabadensis	481.2	15	99.8	Chandra & Tandon (1963)
	Pestalotia psidii	305.2	12	91.8	Ghosh et al. (1966)
	Phoma psidii	305.2	08	100	Ghosh et al. (1966)
	Phomopsis psidii	372.2	10	79	Arya (1982) Acc. to Dasgupta & Mandal (1989)
Tomato	Cylindrocladium scoparium	23	10	89.1	Tandon and Jamaluddin (1973)
	Drechslera australiense	25	10	98.1	Kapoor and Tandon (1969)
	Geotrichum candidum	22.3	12	46.8	Jamaluddin et al. (1972)
	Myrothecium roridum	27.2	15	86.7	Jamaluddin et al. (1974)
	Nigrospora oryzae	47	08	100	Reddy et al. (1979)
	Phoma exigua	47.0	08	91.7	Reddy et al. (1979)
	Rhizoctonia solani	47.0	08	100	Reddy et al. (1979)
	Stemphylium vericarium	47.0	08	100	Reddy et al. (1979) Acc. to Dasgupta & Mandal (1989)

ascorbic acid was observed in musambi fruits infected with B. theobromae (Srivastava & Tandon, 1966b). In papaya fruits infected with A. niger, there was 81.3% loss of ascorbic acid (Singh, 1968), however, there was 92.8% loss after 12 days of incubation in musambi fruits infected with B. theobromae. Kapoor and Tandon (1969) found reduction in tomato infected with Drechslera australiense, guava infected with A. niger (Tandon & Jamaluddin, 1973) chilli fruits with Choanephora cucurbitarum (Chahal & Grover, 1972). Chilli fruits infected with R. stolonifer (Tandon et al, 1974). Papaya fruits infected with A. tenuis (Prasad & Verma, 1976) and banana fruit infected with H. specifer (Prasad, 1977).

Tropical fruits are important sources of vitamin C. Ascorbic acid content normally diminishes during storage, which is enhanced by an injury or infection (Dasgupta & Mandal , 1989), fungi differ in the rate and degree of reduction of ascorbic acid (Table No. 6).

ENZYME :

Chesson (1980) has reviewed the role of pectolytic microorganisms or cell free pectic enzymes in relation to tissue maceration during post harvest handling and processing. Abdetal et al. (1986) studied enzyme activities during development of certain citrus fruit rot diseases. Cellulase activity increased

in falvedo, albedo & pulm of naval organes as a result of infection by Geotrichum candidum, Phytophthora citrophthora and Diplodia natalensis, polygalacturonase was detected at higher levels in diseased flavedo and abledo tissues while its activity in the pulp was less than in controls. Catalase was also present following infection, especial in the flavedo. Ascorbic acid oxidase actively was greatly reduced in all fruit parts. Per-oxidase activity in the albedo and pulp was highest after inoculation with P. citropthora and B. theobromae; in the flavedo, it was not affected, Polyphenol oxidase activity in albedo and pulp tissues remained unchanged as a result of P. citrophthora infection and decreased in flavedo.

Kantaishreede et al. (1987) observed the effect of colletotrichum infection on the oxidizing enzymes of different citrus fruits. Changes in activity of polyphenol oxidase, peroxidase, ascorbic acid oxidase and catalase were determined in fruits of 3 species inoculated with G. gleosporioides (Glomerella cingulata). There was a tendency for activity to be greatest in citrus medica, which is less susceptible than C. sinencis or C. reticulata.

Gautam et al. (1987) observed changes in oxidative enzyme activity for 15 days at 3 days intervals in healthy, incised and diseased peel and pulp of citrus medica fruits infected by Thielaviopsis (Ceratocystis) paradoxa. The enzyme studied were : peroxidase, polyphenol oxidiase, catalase and ascorbic acid oxidase. Little difference was found in polyphenol oxidase

Table No. 7: INVIVO PRODUCTION OF ENZYMES BY POST HARVEST PATHOGENS OF PERISHABLES

Host	Organism	Enzyme make-up	Role in pathogenesis	References
Banana	Botryodiplodia theobromae	Peroxidase, Phenol-oxidase (PO), PG, cellulose	PG and cellulose activity not correlated with virulent isolate	Chakraborty and Nandi (1978), Gupta and Vyas (1979) Acc. to Dasgupta & Mandal (1989)
Guava	Gloeosporium psidii	Pectolytic enzymes	Associated with rotting	Midha and Chohan (1967)
	Phytophthora nicotianae	PG, PGTE, Cellulase	Cellulase activity more than PG and PGTE	Prasad & Sinha (1979)
Tomato	Alternaria solani A. tenuis	Pectolytic and cellulolytic enzyme	Associated with disease	Mehta et al. (1974); Daradhiyar (1979)
	Botryodiplodia theobromae	PG, PME, PGTE, PMGTE and Cellulase	Associated with disease	Karkun, Deepak and Ali (1979)
	Botrytis cinerea	PG, endo-PL, Pectic enzymes, protease	Exo-PG in green fruit, endo-PG and endo-PL in	Brown and Adikaram (1983) Acc. to Dasgupta & Mandal (1989)

		ripe fruits; protease, trypsin, and chymotrypsin activity associated with rotting	
Colletotrichum coccodes	Pectolytic enzymes and protease	Endo-PL associated with rotting	Brown and Adikaram (1983)
Corynespora cassiicola	Cell wall degrading enzymes	PG initiates infection and later PMG, PMTE and cellulase associated	Karkun , Deepak and Ali (1978)
Curvularia lunata	PG, PP, C_1 and C_x	Percentage activity of enzymes proportional to incubation period	Daradhiyar (1979)
Curvularia veruculosa	PG, PME, PMGTE, cellulase	PG, PMG, PMGTE responsible for rotting and cellulase activity later on noted.	Karkun, Deepak and Ali (1978)
Cylindrocarpon tokinense	PG, DP, C_1 and C_x	Associated with disease development and enzyme activity dependent on period of incubation.	Daradhiyar (1979)

Cylindrocarpon tokinense	C_1 and C_x	Role in pathogenesis and correlated with incubation period.	Bilgrami and Verma (1978)
Drechslera hawaiiensis	PG and exo-PG	Associated with disease	Reddy and Laxminarayana (1979)
Fusarium roseum	Pectolytic enzymes	Associated with disease development	Acc. to Dasgupta & Mandal (1989) Aulakh (1977)
Glomerella cingulata	Pectic enzymes and proteases	Associated with rotting	Brown and Adikaram (1983)
Oospora lactis	Pectolytic enzyme	Associated with rotting	Aulakh (1977)
Phoma destructiva	PG, PMG, PME, PTE, PGTE	Associated with rotting	Aulakh (19777) Hazija and Batra (1981)

C_1 = Fraction of cellulase converting native cellulose into shorter linear polyanhydroglucose chains;

C_x = Carboxymethylcellulase

DP = Depolymerase

PG = Polygalacturonase

PTE = Pectin transeliminase (= PMTE)

PGTE = Polygalactotranseliminase

PO = Phenoloxidase (= Polyphenoloxidase)

PME = Pectin methyl esterase

PMG = Polymethylgalacturonase

PP = Protopectinase

PMTE = Pectin methyl transeliminase

PL = Pectin lyase (= PTE/PMTE)

activity in incised and healthy tissues, but more in peroxidase, catalase and ascorbic acid oxidase activity. Low et al. (1989) found the reduction in glucose content in potatoes with glucose oxidase.

Table No. 7 shows the invivo production of enzymes by post harvest pathogens of perishables.

PLAN OF WORK

1. Isolation and identification of fungi and bacteria from fruits and vegetables (Cruickshank, *et al.*, 1973; Riker & Riker, 1936) from warehouses and market.

2. Pathogenicity of different microorganisms on respective fruits and changes in ascrobic acid, amino acid, proteins and carbohydrate contents.

3. Inoculation of fruits with two or more microorganisms simultaneously, sequentially and consequential biochemical changes.

4. Inoculation of fruits with pathogenic microorganism and with microorganisms antagonistic to pathogenic ones and development of resultant disease and biochemical changes.

5. Growths of pathogenic microorganism in the presence of antagonistic microorganisms *in vitro*. Effect of culture filtrate on growth of pathogenic microorganisms.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Fresh vegetables and fruits purchased will be collected and kept in sterilised containers. Great care will be taken during transportation and handling.

ISOLATION OF MICROORGANISMS

A sterile normal saline solution dipped swab will be passed through various area of the resultant inoculum will be prepared and streaked upon the Nutrient agar, Eosine methylene blue agar, Mac conkey agar, Potato dextrose agar and Sabroud's dextrose medium contained in petri plates. The inoculum will be placed directly in the plates or different dilutions will be made. The plates will be incubated for 24 to 48 hrs. for bacterial infection and 5-10 days for fungal infection. Bacterial flora will be identified on the basis of morphology, staining and physiological activities (Cruickshank et al., 1973). Fungi will be isolated in pure culture by hyphal tip/single spore isolation and will be identified by using keys.

The frequency of fungi/bacteria will be calculated as:

$$\text{Frequency} = \frac{\text{No. of plates containing a particular fungus/ bacteria}}{\text{Total plates poured}} \times 100$$

$$\text{and Relative Abundance} = \frac{\text{Total no. of colonies of a fungus/bacteria}}{\text{Total no. of colonies of all the fungi/ bacteria}} \times 100$$

PREPARATION OF SWABS (Cruickshank et al., 1973)

A swab usually consisted of cotton tightly wrapped round one end of a wooden stick 16 cm. long. It was fitted to a tube and was autoclave at 15 psi for 15 minutes These swabs will be rubbed aseptically on the surface of vegetable and fruits.

For isolation of pathogens, the diseased fruits will be thoroughly washed with tap water and then the surface will be sterilized with 0.1% $HgCl_2$ solution for 1 to 2 minutes. After repeated washing with sterile distilled water, diseased lesions will be removed and will be transferred in sterilized petridishes containing PDA and nutrient agar. Before transfer of the diseased tissue the petridishes will be incubated at 28±1°C. The bacterial colony and fungal hyphae growing out of the diseased tissues will be transferred to fresh petridishes containing PDA and NA. The pure cultures will be maintained on PDA and NA slants.

CONSTITUENTS OF MEDIA TO BE USED FOR IDENTIFICATION OF BACTERIA

1. NUTRIENT BROTH : (Sydney and William, 1982)

Broth peptone	- 5 g
NaCl	- 5 g
Yeast extract	- 1-5 g
Beef extract	- 1-5 g

Distilled water	-	1 litre
pH	-	7.2 ± 0.2

The above ingredients will be mixed together, adjusted to pH 7.2

2. NUTRIENT AGAR : (Loc.cit)

Peptone	-	5 g
Yeast extract	-	3 g
NaCl	-	5 g
Agar	-	15 g
Distilled water	-	1 litre
pH	-	6.8

The above ingredients will be mixed together and autoclaved.

3. MAC CONKEY AGAR (Loc.cit)

Peptone	-	14 g
Protease peptone	-	03 g
Lactose	-	10 g
Bile salts	-	1.5 g
Sodium chloride	-	05 g
Agar	-	13.5 g

Neutral red	-	0.03g
Crystal violet	-	0.001 g
Distilled water	-	1 litre
pH		± 7.1

4. **POTATO DEXTROSE AGAR MEDIUM** (Finegold & Martin, 1982)

Potato (peeled & sliced)	-	250 g
Dextrose	-	020 g
Agar	-	015 g
Rose bengal	-	0.32 g
Distilled water	-	1 litre

The potatoes will be boiled in water for 15 minutes, filtered through cotton cloth and made upto volume with water. Added dry ingredients and agar will be dissolved. No pH adjustment will be required .

5. **SABOURAUD DEXTROSE AGAR** (Larone, 1976)

Dextrose	-	20 g
Peptone	-	10 g
Agar	-	17 g
Distilled water	-	01 litre
pH	-	5.6

6. **RICHARDS MEDIUM** (Riker and Riker, 1936)

Potassium nitrate	-	10 g

Potassium dihydrogen phosphate	- 05 g
Magnesium sulphate	- 2.5 g
Ferric chloride	- 0.02 g
Sucrose	- 50 g
Distilled water	- 01 litre

7. CZAPEK'S MEDIUM (Loc.cit)

Sucrose	- 30 g
Sodium nitrate	- 02 g
Potassium dihydrogen phosphate	- 01 g
Magnesium sulphate $7H_2O$	- 0.5 g
Potassium chloride	- 0.5 g
Ferrous sulphate	- 0.01 g
Distilled water	- 01 litre

8. CITRATE AGAR (Simmon's, 1962)

Agar	- 20 g
NaCl	- 05 g
Magnesium sulphate	- 0.2 g
Ammonium dihydrogen phosphate	- 01 g

Dipotassium phosphate	-	01 g
Sodium citrate	-	02 g
Bromethymol blue	-	0.08 g
pH	-	6.9

9. METHYL RED-VOGES PROSKAUR MEDIUM (Sydney & William, 1982)

Buffered peptone	-	7 g
Glucose	-	5 g
Dipotassium phosphate	-	5 g
Distilled water	-	1 litre
pH	-	6.9 ± 0.2

10. MOTILITY MEDIUM (Loc.cit)

Beef extract	-	03 g
Peptone	-	10 g
NaCl	-	05 g
Agar	-	04 g
Distilled water	-	01 litre

In all the above media, ingredients will be mixed in water and boiled. The media will be autoclaved at 15 lbs pressure for 15 minutes. pH will be adjusted to ± 6.00.

REAGENTS/SOLUTIONS TO BE USED FOR IDENTIFICATION

1. **GRAM'S STAINING SOLUTION** (Hucker's Modification)

(A) (i) **Stock Crystal Violet Solution :**

Crystal violet	-	20 g
Ethanol (95%)	-	100 g

(ii) **Stock ammonium oxalate solution :**

Ammonium oxalate	-	1 g
Distilled water	-	100 ml.

The two solutions will be mixed and allowed to stand for 24 hrs and then will be stored in a glass stoppered bottle.

(B) **Gram's iodine solution :**

Iodine crystals	-	1 g
Potassium iodide	-	2 g

The above chemicals will be mixed in 5 ml of distilled water, and to this solution the following will be added.

Distilled water	-	240 ml.
5% aq. soln. of sod. carbonate	-	60 ml

The contents will be mixed well and will be stored in glass stoppered bottles.

(C) **Decoloriser :**

Ethanol (95%) and acetone will be mixed in a mixture of equal volume.

(D) **Counterstain** (Stock safranins)

Safranin	-	2.5 g
Ethanol	-	100 ml

Working solution : The stock safranin will be diluted as 1:5 or 1:10 with distilled water and be stored in glass stoppered bottle.

The bacteria will be stained as per technique suggested by Sydney and William (1982).

INDOLE PRODUCTION TEST (Sydney & William, 1982)

Reagent :

Paradimethyl amino benzaldehyde	-	10 g
Ethyl alcohol	-	150 ml
Hydrochloric acid	-	50 ml.

p-dimethylamino benzaldehyde will be dissolved in alcohol. To this will be added hydrochloric acid slowly. The organism in the nutrient broth will be tested to indole production by adding 5 drops of kovac's reagent. A deep red colour will indicate the production of indole.

2. **METHYL RED TEST FOR ACID PRODUCTION** (Clark & Lubs, 1975)

Methyl red (0.1 g) will be dissolved in 300 ml of ethyl alcohol (95%) and diluted with distilled water.

Appearance of red colour in culture broth by adding 5 drops of methyl red solution will indicate the presence of acid (pH 4.3).

3. **VOGES PROSKAUR TEST** (Sydney and William, 1982)

Reagent :

The reagent will be prepared by adding alpha naphthol (5% in absolute ethyl alcohol) in KOH (40% containing 0.3% creatinine. To culture grown in broth will be added 0.6 ml of 5% α-naphthol in a absolute ethyl alcohol together with 0.2 ml of 40% Potassium hydroxide creatinine solution. After 10-20 minutes the production of bright orange red colour will indicate the presence of organic acid.

METHODS FOR ESTIMATION OF BIOCHEMICAL CONTENTS

Fresh, stored, preserved and inoculated fruits and vegetables shall be analysed for carbohydrate, protein, amino acid and ascorbic acid content.

The dried pulp will be macerated in ground-glass homogeniser containing acid washed sand and 20 ml of 80% ethanol. Later, the pulp will be boiled in 20 ml of 80% ethanol on a water bath, thrice in order to obtain last traces of organic compounds present. The solution, obtained will be kept at temperature overnight. The solution obtained will be centrifuged at 2,000

rpm for 30 minutes. These studies will be initiated after 3 days of inoculation and will be continued upto 12 days of inoculation.

ESTIMATION OF TOTAL PROTEIN

Proteins present in extracts have to be separated from other interfering substances prior to their estimation.

Proteins will be estimated following the methods of Lowry et al. (1951) by using Folin's reagent.

Reagent A : 2% sodium ions carbonate in 0.1 N NaOH.

Reagent B : 0.5% $CuSO_4$. 5 H_2O in 1% sodium tartarate (prepare fresh).

Reagent C : 50 ml of reagent A and 1 ml of reagent B.

Reagent D : Folin-ciocalteu reagent in the Laboratory.

Reagent E : 1 N NaOH.

Standard curve will be obtained with egg albumin. Albumin (10 mg) will be dissolved in 100 ml of 1 N. NaOH (1 ml) will be diluted to obtain 10 ml by adding more 1 N NaOH. From this 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml will be transferred to test tubes and the volume will be made upto 1 ml. To this 5 ml of the reagent will be incorporated. The mixture will be allowed to stand at room temperature for about 10 minutes. To this will be added 0.5 ml of Folin's reagent and mixed immediately.

After 30 minutes the optical densities will be measured in Bausch and Lomb Spectronic-20 calorimeter at 660 nm against a reagent blank. The amount of protein in the samples will be estimated by matching, the optical density with standard curve.

ESTIMATION OF TOTAL FREE AMINO ACIDS :

Amino acids will be estimated by Moore and Stein (1954) Method.

Ninhydrin Reagent : To 750 ml of methyl cellulose will be added 20 g of ninhydrin and 3 g of hydrindantin. To this solution 250 ml of sodium acetate buffer (pH 5.5) will be added. As soon as the resultant redish reagent becomes yellow, it will be transferred into a dark glass bottle. The reagent will be used within a week.

Standard curve will be obtained by estimating amino acid in a solution of leucine. Initially leucine (10 mg) will be dissolved in 100 ml of distilled water. Aliquotes of 0.1 to 1 ml in different concentrations will be used. The volume of each, if less, will be made to 1 ml. To this will be added 1 ml of ninhydrine reagent. This will be boiled for 15 minutes in water bath. The absorbance will be determined at 570 nm for all the amino acid except for proline and hydroxyproline which will be measured at 440 nm in spectrophotometer.

ESTIMATION OF CARBOHYDRATE

Carbohydrate would be extracted and estimated by using the methods of Yin and Clark (1965) and Dubois et al. (1956) respectively.

Glucose (Analar , 10 ml) will be dissolved in 100 ml of distilled water and 1 ml of this will again diluted to 10 ml Different quantities of the diluted solution i.e. 0.1 to 1.0 ml would be transferred to test tubes and the volume will be made upto 1 ml by adding distilled water. To these solutions, 1 ml of 5% phenol and 5 ml of concentrated sulphuric acid (Analar) will be added. After 10 minutes optical densities will be measured in Bausch and Lomb Spectronic-20 colorimeter at 490 nm against a reagent blank. Graph will be plotted between optical densities and different concentration of the glucose solution. This will serve as a 'standard curve' which will be used for estimation of carbohydrate in solution.

ESTIMATION OF ASCORBIC ACID

Ascorbic acid is estimated by the visual titration method based on the reduction of 2, 6-dichlorophenol dye (Tandon et al. 1974).

The sample will be prepared by blending vegetable/fruit pulp (10 g) in 100 ml of 2% oxalic acid. The pulp will be sieved and transferred to 250 ml volumetric flask giving several washing

2% oxalic acid and the volume will be made to 250 ml, the aliquot will be filtered through the muslin cloth.

Indophenol reagent (Dye: 2-6, Dich-lorophenol Indophenol)

Sodium 2, 6-dichlorophenol indophenol (50 mg) will be added to 150 ml glass distilled water. It will be warmed to dissolved the dye $NaHCO_3$ (40 mg) will be added. The volume will be made to 200 ml by adding glass distilled water. The reagent will be stored in dark glass bottle at 2°C.

Ascorbic acid standard solution :

To 50 ml of 2% oxalic acid will be added .50 ml of ascorbic acid in a 250 ml volumetric flask and volume will be made to 250 ml with oxalic acid. Thus giving 0.2 mg of ascorbic acid in 1 ml.

Standardization :

Indophenol reagent will be standardized before use. Ascorbic acid solution (5 ml) will be pipetted to a white porcelain dish and will be titrated against indophenol dye. The first appearance of the pink colour will be taken as an end point.

Similar procedure will be used for the estimation of the unknown ascorbic acid.

Calculation :

The ascorbic acid content of the extract will be calculated as follows :

IxSxD/Ax100/W = mg of ascorbic acid/100 g of tissue where,

S = mg of ascorbic acid reacting with 1 ml of the reagent.

I = ml of Indophenol reagent used in titration.

D = volume of this extract in ml.

A = the aliquot titrated in ml and

W = the weight of the sample in g.

INOCULATION OF FRUITS AND VEGETABLES :

Healthy ripe fruits and vegetables will be inoculated with test fungus and bacteria separately and in different combinations to ascertain the antagonistic behaviour of the two microorganism. Inoculation of fruits and vegetables by fungus will be done by pin-prick method while with bacterium by spraying the fruits with bacterial suspension after imparting some injury to the host.

Inoculation levels of fungi and bacterium water suspension will be prepared and number of spores per ml of suspension will be counted by haematocytometer. Where the sporulating fungus

is not available, mycelial suspension will be made by with known amount of mycelium. The fruits so inoculated will be incubated in sterile desicators, the bottom of which will be filled with sterile water.

After different intervals the extent of rotting will be calculated by measuring the rotted area.

The pulp will be used for estimating the amino-acid, proteins, carbohydrates, ascorbic acid and enzyme activity.

Appropriate controls will be maintained throughout.

The data will be subject to statistical analysis.

The interaction of different pathogens will also be studied in vitro. The two microorganism will be grown on sterilized czapecks medium contained in sterilized petridishes. These plates will be inoculated with 2 or 3 or 4 microorganism in different combinations on different places, so that all the microorganisms appear to be located at equal distance from the centre of petridish. The growth of the fungi/bacteria will be measured and inhibition zone if any, indicating inhibition of the microorganisms will be noted.

The microorganism indicating inhibitory zones will be tested for inhibitory properties in their culture filtrates. The microorganism will be grown in the media supporting the optimum growth contained in 250 ml Earlemeyer flask. After 15 days for

fungi and after 7 days of growth of bacteria the culture filtrate will be obtained by passing the contents of the flask through filter paper. The filter paper strips dipped in the culture filtrate will be kept in petridishes containing the media on one side and on the opposite side the microorganism will be grown. The inhibition zone if any will be determined.

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